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CHEMICAL AND MICROBIOLOGICAL ASSESSMENT OF RAW CAMEL'S MILK WITH SPECIAL REFERENCE TO SUBCLINICAL MASTITIS MONITORING IN EGYPT

(With 4 Tables)

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التقييم الكيميائى والميكروبيولوجى لالبان الجمال الخام ودراسة التهاب الضرع الغير الظاهرى فى اناث الجمال فى مصر

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تم فحص ١٠١ عينة لبن خام من نوق سليمة ظاهريا وقد أخذت العينات من الارباع الحلابة فى زجاجات معقمة لتحليلها ميكروبيولوجيا وكيميائيا ولمعرفة نسبة الاصابة بالتهابات الضرع الغير الظاهرى بين اناث الجمال واسبابها الميكروبيولوجية. وقد دلت نتائج اختبار الكاليفورنيا (CMT) ان ٥١ عينة (٥٠,٥ %) كانت سلبية و ٥٠ عينة (٤٩,٥ %) كانت موجبة وكانت نسب CMT+ ، CMT++ ، CMT+++ بين العينات الايجابية هى ٢٦,٧% ، ١٢,٩ % ، ٩,٩ % على الترتيب. وكان متوسط عدد الخلايا الجسيمية فى عينات اللبن الايجابية والسلبية لاختبار الكاليفورنيا ٣٠٤ x 10^3 ، ٦٩ x 10^3 على الترتيب. وهذا مايؤكد الارتباط الشديد بين اختبار الكاليفورنيا (CMT) وعدد الخلايا الجسيمية (SCC) فى كلتا العينات الايجابية والسلبية لاختبار الكاليفورنيا. أظهرت نتائج التقييم الميكروبيولوجى ان أهم المسببات البكتيرية لالتهاب الضرع غير الظاهرى فى اناث الجمال هى ميكروب المكور العنقودى الموجب لاختبار التجلط (**CPS**) والسالبة منها (**CNS**) والميكروبات القولونية (**coliforms**). كما اوضحت النتائج ارتفاع العدد الكلى للميكروبات الهوائية والميكروبات القولونية والعدد الكلى للخمائروالعفن وكذلك عدد ميكروب المكور العنقودى الموجب لاختبار التجلط (CPS) فى عينات اللبن الخام للجمال. وقد اظهر التحليل الاحصائى لنتائج عينات اللبن السليمة والمصابة بالتهاب الضرع غير الظاهرى وجود فروق معنوية فيما بين متوسطات فى كل من: نسبة الدهون – نسبة البروتين– نسبة اليوريا – نسبة الأجسام

الصلبة الكلية – نسبة الأجسام الصلبة غير الدهنية– عدد الخلايا الجسيمية – العد البكتيرى الكلى (TBC)– العدد الكلى للمكورات العنقودية – عدد الميكروبات القولونية – عدد الخمائر.

SUMMARY

One hundred and one quarter camel's milk samples from apparently healthy lactating she camels were collected in sterile bottles in order to be evaluated microbiologically and chemically and to distinguish the prevalence of subclinical mastitis in camel and their microbial causes. Among the positive CMT samples, the incidence of CMT (+) score, CMT (++) score and CMT (+++) score were 26.7%, 12.9 % and 9.9%, respectively. The mean SCC of negative and positive CMT milk samples were 69 $x10^3$ ± 7.32 & 304 $x10^3$ ± 2.52, respectively. The study confirmed the high correlation between CMT and SCC in both negative and positive CMT camel's milk samples. The most common causes of subclinical mastitis in examined camel milk samples were *CPS*, *CNS* and coliforms. Moreover, it has been reported that raw camel's milk has high levels of total aerobic count, coliform counts, total yeast and mould counts and coagulase positive staphylococci. The analysis of variance (ANOVA) clarified the significant differences of means between normal and mastitic milk at ($p < 0.05$) in fat %, protein%, urea mg%, total solids %, SNF%, SCC, TBC, staphylococci count, coliform count and yeast counts. In conclusion, fresh camel milk is a perfect and highly nutritional food if produced under acceptable hygienic measures to be free from any human health hazards due to subclinical mastitis or post milking contamination.

Key words: *Milk, raw camel's milk, subclinical mastitis, CMT.*

INTRODUCTION

Camels are one of the most valuable food resources for human in arid and semi-arid regions, which provides milk almost the year better than any other lactating animals held under the same harsh conditions that are severely affected by heat, scarcity of water and feed (Park and Haenlein, 2006). Their daily yield between 3-10 Kg in a lactation period of 12 – 18 months is common (Farah *et al.*, 2007).

In the last years, consumption of camel milk among the urban population was increased dramatically (Chaibou, 2005; Farah *et al.*,

2007) with the increasing suggestions of its therapeutic values (Agrawal *et al.*, 2005). These fast growing demand for raw camel milk all over the world magnify the significance of the microbial evaluation of raw camel's milk.

As camel milk is usually consumed in its raw state thus the possible presence of (Semereab and Molla, 2001) pathogenic bacteria may constitute a public health hazards to consumers.

Mastitis is a relatively infrequent disease in *Camelidae* compared with cattle and sheep that explained the few data concerning the etiology, occurrence of mastitis in *Camelidae* (Abdel Gadir *et al.*, 2006). There is no doubt that mastitis has both an extreme zoonotic and economic importance constituting multiple hazardous effects on human health and animal production. (Hegazy *et al.*, 2004; Al-Majali *et al.*, 2008). There are evidences that Coagulase positive as well as coagulase negative staphylococci, *Streptococcus spp.* and *Escherichia coli* are the major bacterial causes incriminated in camel mastitis. (Younan, 2004; Kotb *et al.*, 2010).

Therefore, this work was carried out to monitor camel milk quality as well as detection of udder pathogens that causing mastitis using California mastitis test (CMT), determination of somatic cell counts (SCC), biochemical and microbiological examination of camel milk samples from normal and mastitic she camel.

MATERIALS and METHODS

1- Study population: The study was carried out on camel – small holders - farms in different regions in Egypt. The camel milk is produced in traditional way by hand milking, handled and transported under low hygienic measures and consumed in a raw state.

2- Samples: A number of 101-quarter milk samples were taken from apparently healthy lactating she-camels. Each sample was collected in clean, sterile and dry McCartney glasses in duplicate. One sample was examined for milk composition while the other was used in bacteriological studies. The milk samples were preserved in ice box after sampling till examined in the laboratory.

3- Field test (California Mastitis Test, CMT): According to APHA (2004) for detection of subclinical mastitis in lactating she-camels, CMT was performed on individual milk samples collected from each quarter of all examined she-camels. Depending on the amount of gel formation,

samples were assigned into the following categories: negative or positive reaction in 4 grades (+, ++, +++ and ++++).

4- Somatic Cell Count (SCC): SCC was done automatically using SOMA-COUNT 150 from Bentley. The SCC measures the number of white blood cells and udder squamous epithelial cells in milk that were present in large number in case of subclinical mastitis (Zecconi *et al.*, 2002)

5- Bacteriological examination: All the collected camel milk samples were subjected to microbiological analysis for:

a) Total bacterial count (TBC): according to BAM, on line (2009).
b) Isolation and identification of staphylococci: according to BAM, on line (2009).
c) Coliform count: according to Collins *et al.* (1995) and BAM, on line (2009).
d) Total yeast and mould counts according to APHA (2004).

6- Measurements of milk constituents: Using infrared milk analyzer-150, from Bentley, the following milk constituents were estimated in all examined camel milk samples: fat %, protein %, lactose %, urea mg %, total solids %, and SNF %.

7- Statistical analysis.

RESULTS

Table 1: Statistical analytical results of CMT and SCC and their correlations in the examined camel's milk samples.

	No of examined samples		Mean SCC (x 1000)	Intervals		
	No.	%		< 200	200 – 400	> 400
CMT negative	51	50.5	69 ± 7.32 (10 - 210)	49 (48.5%)	2 (1.99%)	0
CMT (+)	27	26.7	304 ± 2.52 (110 - 658)	12 (11.9%)	14 (13.9%)	1 (0.99%)
CMT (++)	13	12.9		6 (5.91%)	6 (5.91%)	1 (0.99%)
CMT (+++)	10	9.9		0	1 (0.99%)	9 (8.91%)
TOTAL	101	100		67 (66.31%)	23 (22.79%)	11 (10.9%)

Table 2: Prevalence of bacterial infections in the examined camel's milk samples

	TBC		Coliform count		CPS		CNS		Total Yeast & Mould counts	
	$< 10^5$	$> 10^5$	< 500	> 500	-ve	-ve	< 500	> 500	-ve	+ve
CMT negative milk samples	19 18.8%	32 31.7%	42 41.6%	9 8.9%	35 34.7%	16 15.8%	23 22.8%	28 27.7%	9 8.9%	42 41.6%
CMT positive milk samples	13 12.9%	37 36.6%	16 15.8%	34 33.7%	20 19.8%	30 29.7%	24 23.8%	26 25.7%	6 5.9%	44 43.6%

Table 3: Statistical analytical results of milk parameters in the examined camel's milk samples

	No. of examined samples	FAT %	Protein %	Lactose %	Urea mg%	Total solids %	SNF %
CMT negative milk samples	51	2.8 ± 0.11	2.66 ± 0.05	3.95 ± 0.09	21.92 ± 0.71	9.96 ± 0.16	7.45 ± 0.16
CMT positive milk samples	50	2.1 ± 0.10	2.40 ± 0.06	3.22 ± 0.06	18.72 ± 0.99	9.22 ± 0.09	6.47 ±0.13

Table 4: Analysis of variance (ANOVA) between different examined parameters of normal & mastitic camel milk samples

Parameters	Mean of normal samples	Mean of mastitic samples	LSD
Fat %	2.8	2.1	0.45*
Protein %	2.66	2.40	0.001*
Lactose %	3.95	3.22	0.0
Urea mg%	21.92	18.72	0.013*
Total solids %	9.96	9.22	0.03*
SNF %	7.45	6.47	0.03*
SCC (x 1000)	69	304	0.01*
TBC	463×10^7	132×10^9	0.3*
Total Staph count	80×10^4	78×10^6	0.045*
Coliform count	481	1460	0.023*
Total Yeast & Mould counts	52.4×10^6	811×10^6	0.49*

*The mean difference is significant at the $p < 0.05$ level

DISCUSSION

The most common forms for consumption of camel milk are either fresh (raw milk) or fermented. Due to the increased commercialization of camel milk and fast growing demand for raw camel milk in Egypt, a better knowledge on its quality and production with special reference to mastitis was needed to be assessed.

CMT gives a sharp discrimination between normal and subclinical mastitis milk samples and it is considered the most important screening field test in predicting camel udder infection status comparatively to somatic cell count (SCC) (Abdu-Rahman, 1996; Sargeant *et al.*, 2001).

The data represented in Table 1 showed that 101 examined camel milk samples were classified into 51 (50.5%) CMT negative samples and 50 (49.5%) was CMT positive samples. Among the positive samples, the highest incidence was recorded in CMT (+) score as 27

(26.7%) and the lowest in CMT (+++) score as 10 (9.9%), while CMT (++) score was 13 (12.9 %). These results were higher than that recorded by Mody *et al.* (1998); Kotb *et al.* (2010) and lower than those reported by Hawari and Hassawi (2008); Seifu and Tafesse (2010).

The obtained results confirmed the positive correlation between CMT with the presence of subclinical mastitis in camel milk and support the use of CMT as useful screening test for detection of mastitis in camel as reported by Abdu-Rahman (1996); Radostitis *et al.* (2005)

The mean SCC of negative and positive CMT milk samples were 69 $x10^3$ ± 7.32 & 304 $x10^3$ ± 2.52, respectively (Table 1). The highest frequency distribution of SCC in CMT negative samples were less than 200$x10^3$ cells/ ml (48.5%), while in case of whole CMT positive milk samples (either +, ++ or +++ CMT), 21 of them had SCC ranged 200 – 400 $x10^3$ cells/ ml and 11 milk samples their SCC were more than 400 $x10^3$ cells/ ml, that reflected on the high mean SCC in CMT positive milk samples than that of CMT negative samples (Table 1).

The obtained results of SCC in CMT negative milk samples agree to some extent with that recorded by Saleh and Faye (2011), while those of CMT positive milk samples were lower than the SCC that reported by Woubit *et al.* (2001); Tuteja *et al.* (2003); Wernery (2007) and higher than that indicated by Saleh and Faye (2011).

This study confirmed that SCC is one of the screening procedures and good indicator for both clinical and subclinical mastitis in camel udder infection (Merin *et al.*, 2004). However the interpretations of results were problematic because the basal levels of SCC and their physiological variations in camel milk are still not yet established (Abdu-Rahman, 1996).

Although Eberlein (2007) was pointed out that an increase in the SCC to more than 300$x10^3$ cells/ ml is considered to be an indication of udder infection in camel, Merin *et al.* (2004) indicated that the values of SCC in infected camel udder are lower if compared with other ruminants (308 $x10^3$ cells/ ml in infected quarter and 118 $x10^3$ cells/ ml in normal quarter).

Moreover, the illustrated results in Table 1 provided an opportunity to confirm the high correlation between CMT and SCC and they were dependant to large extent in both normal and mastitic milk. These results were parallel with those reported by Woubit *et al.* (2001); Wernery (2007); Saleh and Faye (2011).

The assessment of microbiological examination of camel milk samples revealed the pathogenic microorganisms that incriminated in

camel mastitis (either clinical or subclinical), the hygienic measures that applied during milking as well as the bacterial contamination of consumed camel's milk that had public health hazards especially when consumed as raw milk as usual.

Generally, microorganisms can gain access to milk through their colonization in the teat canal, through an infected udder (clinical or subclinical mastitis) or as post milking contaminants. So, the microbiological evaluations of the collected milk samples were done in parallel three axes: evaluation of hygienic quality of raw camel's milk, detection of public health hazard organisms e.g. *E.coli,* staphylococci spp. and yeast and mould as well as total bacterial count and detection the commonest bacterial agents causing subclinical and clinical mastitis in camel.

It is worth to mention that there are no microbiological standards concerning camel milk, therefore microbiological limit values for cow milk were used to assess the quality of camel's milk. (El-Ziney and Al-Turki, 2007). The microbial results of examined camel milk samples were compared with parameters laid down by European Union Standard Commission (Anonymous, 1992).

Table 2 pointed out the prevalence of isolated mastitis pathogens from examined camel milk samples. Staphylococci, either coagulase positive staphylococci *(CPS)* or coagulase negative staphylococci *(CNS)* were frequently isolated from all the examined samples (Kloos and Schleifer, 1986). The prevalence of *CPS* were 15.8 and 29.7% in CMT negative and positive milk samples, respectively, while, the percentage of isolated *CNS* were higher than the permissible limits (500 cfu/ml) which were 27.7 and 25.7 % in CMT negative and positive milk samples, respectively.

The percentage of isolated *CPS* in normal milk samples was higher than that reported by El-Jakee (1998) (5 %) and Chaffer *et al.* (2000) (8.8%) but lower than that recorded by Abdel Gadir *et al.* (2006) (24.6 %). The presence of staphylococcal pathogens in milk indicate contamination of the milk from skin, mouth or the nose of the food handlers or milkers (FAO, 1992).

The isolated *CPS* and *CNS* from CMT positive camel's milk samples confirmed that both pathogens is considered the most common causes of clinical and subclinical mastitis in dromedaries as proved by Sena *et al.* (2000); Abdel Gadir *et al.* (2006). In Egypt, El-Jakee, (1998) reported that *S. aureus* is one of the most common mastitis pathogens in she camels. Additionally, Abdu-Rahman (1996) demonstrated that *CNS*

and *S. aureus* represented by 61.1 and 38.9 % of the total isolates, respectively, which were considered as the main cause of mastitis in camel.

Barbour *et al.* (1985); Younan (2004) stated that *S. aureus* can produce heat stable enterotoxins which are not inactivated during pasteurization of milk or production of milk products which can provoke food intoxication.

The existence of one of the environmental mastitis pathogens e.g. coliform organisms in milk samples coincide with the insufficient hygienic conditions during milking and further handling processes as well as the fecal contamination of the milk (Bülte, 2004). Moreover, isolation of *E. coli* implies a risk that other enteric pathogens may be present in the examined milk sample (Wernery, 2007).

Inspection of Table 2 revealed that coliform organisms were detected in high counts more than 500 cfu/ml, from 8.9 % and 33.7% of the CMT negative and positive milk samples, respectively. Nearly similar results were recorded by Omer and El-Tinay (2009), while lower values were reported by Wernery (2007) and higher values were noted by Benkerroum *et al.* (2003); El-Ziney and Al- Turki (2007).

Presence of coliforms in camel's milk constitutes a public health concern and it is epidemiologically significant that not only for animals but also for humans. The occurrence of coliforms in milk may therefore be indicator of fecal pollution with possibility of existing associated pathogens (Mossel, 1982).

The high incidence of total bacterial count (TBC) in examined samples (31.7 % and 36.6 %) in CMT negative and positive samples, respectively, could be explained by Wallace (2008) who mentioned the same results and attributed that to mastitis that potentiate the shedding of large numbers of microorganisms into milk. Also, Bramley and McKinnon (1990); Murphy (1997) debated the influence of mastitis on TBC and found that it depend on the type of bacteria, the stage and the degree of infection in the herd.

The higher recorded figures of TBC were agreed with those noticed by Bramley and McKinnon (1990); Hawari and Hassawi (2008) who mentioned that the highest percentage (54.08%) of CMT positive samples was with TBC higher than 3×10^5.

Mycotic mastitis is relatively uncommon in camels (Suheir *et al.*, 2005). The rate of fungal isolation in the present study is considered higher than that encountered by Amel (2003); Suheir *et al.* (2005). This may be explained by what recorded in most studies concerning animal

mastitis which indicated that fungi are not considered as the primary cause of mastitis, while it is usually considered as environmental contaminants related to poor hygienic conditions (Spanamberg *et al.* 2004).

Table 3 shows the effect of udder infection on milk constituents (fat %, protein %, lactose %, urea mg %, Total solids % and solid not fat %). The mean values of them in normal (CMT negative milk) camel's milk were 2.8 %, 2.66%, 3.95%, 21.92 mg %, 9.96 % and 7.45%, respectively. In subclinical mastitic camel` milk samples, the concerning values were 2.1%, 2.4%, 3.22%, 18.72 mg%, 9.22 % and 6.47%, respectively.

The comparison of results in CMT negative milk samples with those of mastitic ones we found that the values of the later were less than that of normal ones. Thus confirm that inflammatory reaction caused by infection in mammary tissues mostly associated with reduction in milk yield as well as changes in its chemical composition due to cellular damage (Frank and James, 2000). The changes in milk composition are also due to in part to impairment of the secretory process, for example the reduced fat content, to a greater extent, they reflect the changed permeability of the secretory tissue. The main result is a diffusion of lactose and potassium from milk into the blood stream, which is matched by an increased transudate of blood plasma into the milk raising the sodium and chloride content. (Sloth *et al.*, 2003; Radostitis *et al.*, 2005).

Moreover, hydration status as well as type of forages eaten by the animal can affect the milk parameters as fat, protein with special reference to urea content of milk (Yagila and Etzion, 1980). In addition they are varied with season (Haddadin *et al.*, 2007), stage of lactation (El-Amin, 1979) and pregnancy (Rodriguis *et al.*, 1985)

The analysis of variance (ANOVA) represented in Table 4 compare the mean values of CMT negative and positive milk samples and clarified the significant differences of means at ($p < 0.05$) in fat %, protein%, urea mg%, total solids %, SNF%, SCC, TBC, staphylococci count, coliform count and Yeast and moulds counts.

The interpretation of low mean values in mastitic milk samples (CMT positive) than normal milk samples (CMT negative) in protein%, lactose % and consequently TS% and SNF% are due to damage occurred in mammary epithelial synthetic cells of the infected udder by microbial toxins due to mastitis.

The higher mean values of total staphylococci count (CPS and CNS), coliform counts in examined mastitic milk samples as well as

SCC indicate that these pathogens are considered the main causative agents of subclinical mastitis in the present study.

In conclusion, the results of chemical parameters of camel's milk revealed that she camel produces nutritious milk for human consumption. While bacteriological results showed that bacterial contamination and consequently the milk quality were influenced by poor hygienic conditions during milking and handling as well as post milking environmental contaminants rather than mastitis pathogens from infected udder. Lacking of poor cooling and storage with ambient summer temperature in Egypt are also factors magnitude the problems of the bacterial contamination.

Finally, the present study thumbs two very important findings, the first that camel's milk is produced in Egypt under low hygienic measures increasing the possibilities of mastitis in lactating she camels, while the second that raw milk may contain very dangerous human health hazard organisms even from CMT negative milk samples. So, the hygienic control measures targeting to improve the raw camel milk quality and its production as well as the health of lactating she camels.

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